FEATURE

Environmentally-benign reaction chemistry using critical fluids — process implications

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Reactions conducted in pressurized fluid media (supercritical fluids) have several distinct advantages in terms of speed, selectivity, and product quality. Using carbon dioxide under high pressure, GRAS (Generally Regarded as Safe) solvents or hot pressurized water also allows compliance with environmental and worker safety regulations. Reactions in such media can be conveniently combined with extraction or fractionation steps, and used in both processing and analytical chemistry applications. This paper describes the implications for lipid processing; applications in lipid analysis will be examined in a further paper in the October 2001 issue of Lipid Technology Newsletter.

Introduction

Increasing environmental regulation of both industrial processes and laboratory environments has given rise to the concept of 'green' technologies which have an environmentally-benign effect. Of particular concern are the use of potentially toxic organic solvents for unit processes such as extraction, fractionation and reactions. In recent years, pressurized gases or fluids, called supercritical and subcritical fluids, have been employed as alternatives to organic solvents in the above processes. For example, supercritical fluid extraction (SFE) using supercritical carbon dioxide (SC-CO_2) has been used as an alternative to organic solvents such as hexane, methylene chloride, and benzene to extract a wide variety of lipids. Likewise, in the last decade, SC-CO_2 has been implemented as a replacement solvent for organic solvents used in the classic Soxhlet extraction, particularly for the analysis of the fat/oil content of various food and agricultural products.

In this brief review, the use of critical fluids as a medium for conducting reactions on lipid substrates is presented. There are numerous advantages to using a pressurized gas or fluid at high density for synthetic purposes, such as:

- improvement in mass transfer of reactants and products;
- control of reactants and product solubility;
- the use of lower reaction temperatures;
- the control of the reaction rate by manipulating pressure; and
- alteration of the final product distribution or characteristics.

Because of the relatively high solubilities of lipids in SC-CO_2, and the miscibility of oils and fats in high-temperature pressurized water, these two media can be good solvents in which to conduct lipid chemistry. In addition they meet the 'green' criterion and can be discharged into the environment with a relative benign effect.

At the National Center for Agricultural Utilization Research (NCAUR), an Agricultural Research Service laboratory of the US Department of Agriculture, our group has been investigating the use of the above media for approximately ten years. This work has included the coupling of enzymatic catalysts (lipases) in the presence of SC-CO_2 to provide a completely 'green' synthesis approach, as well as eliminating the use of inorganic catalysts altogether by reacting lipid

Table 1. Reactions on lipid substrates conducted in supercritical carbon dioxide (SC-CO₂), combined supercritical carbon dioxide and hydrogen (SC-CO₂/H₂), or subcritical water (Sub-H₂O). Non-Cr = T-4489 chromium-free catalyst (obtained from United Catalysts, Louisville, Kentucky, USA).

Type of reaction	Reaction medium	Catalyst	Lipid substrates
Esterification	$SC\text{-}CO_2$	Lipase	Simple alcohols/acids
Esterification	$SC\text{-}CO_2$	Lipase	Fatty acids/alcohols
Transesterification	$SC\text{-}CO_2$	Lipase	Soybean oil/methanol
Transesterification	$SC\text{-}CO_2$	Lipase	Ferulate esters/triolein
Transesterification	$SC\text{-}CO_2$	Lipase	Chiral esters
Glycerolysis	$SC\text{-}CO_2$	Lipase	Soybean, corn oils
Glycerolysis	$SC\text{-}CO_2$	None	Vegetable oils
Interesterification	$SC\text{-}CO_2$	Lipase	Soybean, palm oils
Hydrogenation	$SC\text{-}CO_2/H_2$	Ni	Soybean oil
Hydrogenation	$SC\text{-}CO_2/H_2$	T-4489	Soybean oil
Transesterification + H_2	$SC\text{-}CO_2/H_2$	T-4489/lipase	Soybean oil
Hydrolysis	$Sub\text{-}H_2O$	None	Vegetable oils

substrates in $SC\text{-}CO_2$ or subcritical water. Several examples of the synthetic options are presented in the following sections. The applications of such reactions in analytical chemistry for sample preparation and derivatization will be described in a further paper in the October 2001 issue of *Lipid Technology Newsletter*.

Enzymatic-initiated reactions in SC-CO₂

The chemical literature provides many examples of the use of lipases in conjunction with $SC\text{-}CO_2$ to synthesize a plethora of compounds. Table 1 lists reactions on lipid substrates that we have successfully completed in our laboratory. These have

included simple esterifications, transesterifications, glycerolysis, interesterification, hydrogenation, and hydrolysis reactions.

It is critical that the conditions should be optimized with respect to maintaining the enzyme's activity and lifetime. Typically in the presence of $SC\text{-}CO_2$, pressures from 15–40 MPa and temperatures ranging from 50 to 80°C are consistent with sustaining lipase activity and use. Critical to the use of these enzymes is the maintenance of a low level of water with the enzyme to sustain its conformation in the presence of $SC\text{-}CO_2$. For example in the methanolysis of soybean oil, a level of 0.1% by volume of water in $SC\text{-}CO_2$ at 24 MPa and 50°C is sufficient to prevent denaturation of the enzyme. However, additional water can prove inhibitory to facilitating esterification or transesterification reactions and it is fortuitous that $SC\text{-}CO_2$ has a low solubility for water.

Novozyme 435, a lipase made by Novo Nordisk and derived from *Candida antarctica*, has proven excellent for use under supercritical conditions, and all of the enzyme-initiated reactions listed in Table 1 have used this enzyme. (M.W. Christensen of Novozymes A/S, Denmark, described developments in lipase-catalysed interesterification in the April 2001 issue of *Lipid Technology Newsletter*, pages 33–37.) Simple esters such as propyl laurate can be made in under 25 minutes at nearly 100% yield using this lipase. We have extensively used Novozyme 435 in synthesis of the
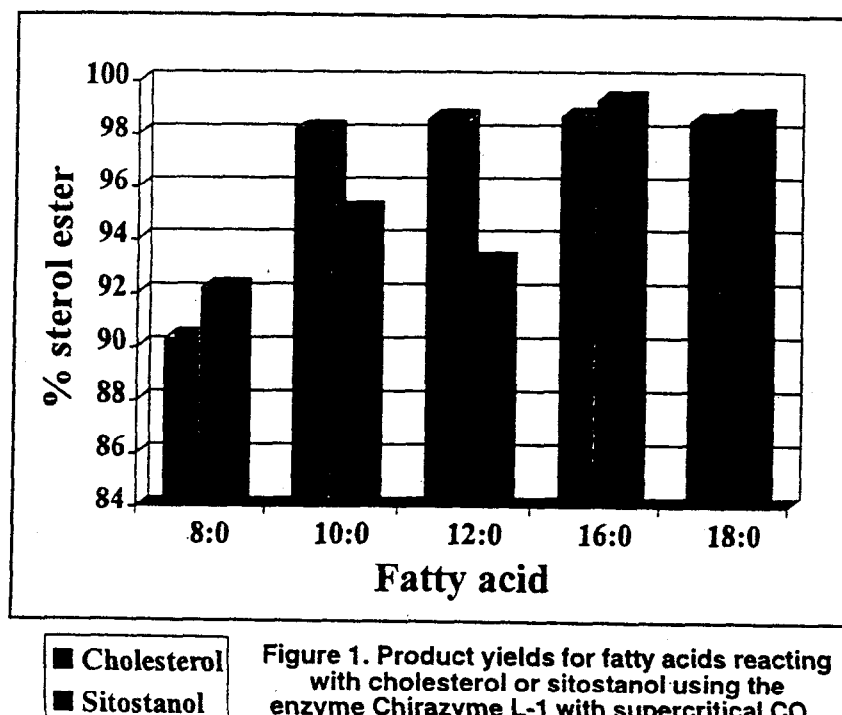
Figure 1. Product yields for fatty acids reacting with cholesterol or sitostanol using the enzyme Chirazyme L-1 with supercritical CO_2.

■ Cholesterol
■ Sitostanol

methyl esters of fatty acids (FAME) derived from various oilseed and food-related products. The fatty acid distributions of lipase-derived FAME synthesized in the presence of $SC-CO_2$ are in excellent agreement both qualitatively and quantitatively with those made using sodium methoxide or BF_3 as catalyst.

Various other esterifications have been successfully conducted using Novozyme 435 as a catalyst. These have included the synthesis of flavour esters and reactions of vegetable oils with diols. Recently, the production of sterol esters has been accomplished using Chirazyme L-1 with $SC-CO_2$. As illustrated in **Figure 1**, fatty acids of various chain lengths have been reacted with sterols, such as cholesterol and sitostanol. Ester yields were consistently above 90% for C_8-C_{18} saturated fatty acids reacting with the sterols at 27.6 MPa and 50°C. Of particular interest were the >98% yields achieved for the C_{16} and C_{18} sitostanol esters which can be utilized in functional food margarine formulations to lower cholesterol levels in humans.

Glycerolysis reactions have also been successfully run in the presence of $SC-CO_2$ using Novozyme 435. Here the reaction takes place in a multiphase system consisting of a large excess of glycerol or diol and an acid functional reactant. One variant of this reaction is conducted in a stirred reactor using only vegetable oil, glycerol and CO_2, and exhibits a yield maximum at 20 MPa **(Figure 2)**. The monoacylglycerol content of the final mixed acylglycerol product is 35–45% by weight for six different commodity vegetable oils. The final product is also white in colour, an improvement over the darker-coloured products obtained using alkaline earth catalysts. The same mixed acylglycerols can also be produced by using a supported lipase catalyst in conjunction with $SC-CO_2$ in a flow reactor. However
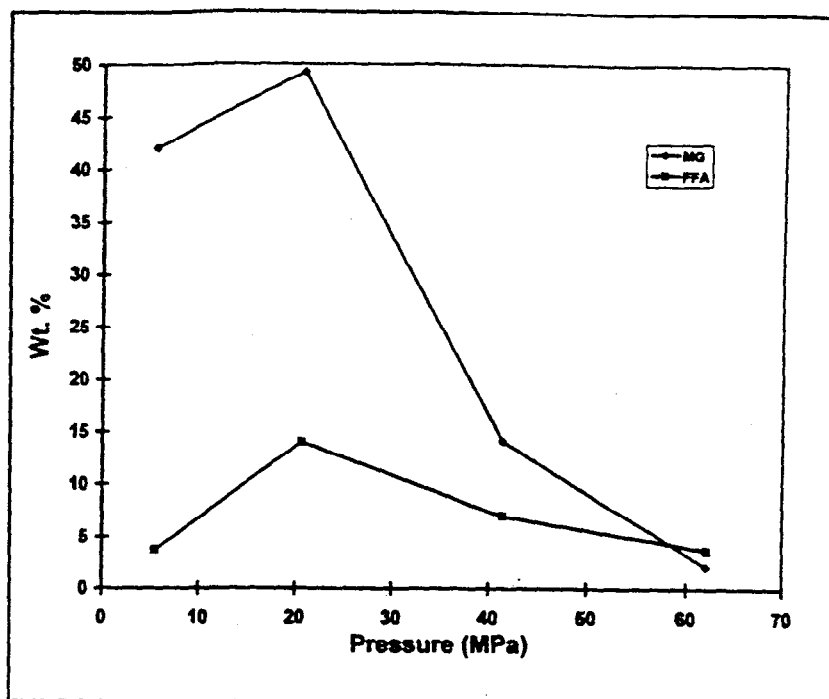

Figure 2. Yield (wt%) of monoacylglycerol (MG) at four different pressures from a multiphase glycerolysis reaction using Novozyme 435 in supercritical CO_2 at 250°C, glycerol/soybean oil ratio of 25, and 4% H_2O added to glycerol.

in this case, by controlling the water content of the glycerol, monoacylglycerol yields as high as 85% can be attained. Again, the synthetic product is superior in colour to commercial products, which use metallic salt catalysts, and does not require removal of any catalyst after completion of the reaction.

Fats and oils can also be randomized (interesterified) using $SC-CO_2$ with Novozyme 435. Here the objective is to produce a randomized mixed acylglycerols product that has the same functionality as a hydrogenated fats/oils composition for use in products such as margarine formulations. Oils or fats are typically dissolved in $SC-CO_2$ at 27.6 MPa and 65°C, and then passed over the immobilized lipase to produce the desired end product. The resultant graph plots for solid fat content with temperature **(Figure 3)** for palm olein and a high-stearate soybean oil (HS-1) randomized in the $SC-CO_2$ flow reactor compare favourably with the values for a

typical hydrogenated oil. We further found that the fat dropping point was dependent on the catalyst concentration, yielding an inverse dependence on the throughput of the dissolved oil across the supported lipase bed. High-performance liquid chromatographic (HPLC) analysis of the randomized and initial oils showed only small changes in the tri-unsaturated, di-unsaturated, di-saturated, and tri-saturated triacylglycerol species.

Hydrogenation under supercritical conditions

Hydrogenation of oils and lipids can also be achieved under supercritical fluid conditions, in other words conditions in which the oil or lipid is dissolved in a binary fluid mixture consisting of either H_2 + $SC-CO_2$ or of H_2 + SC-propane. Harrod and coworkers at Göteborg University (Sweden) have shown that very high yields can be achieved in hydrogenating oils or oleochemicals at high space
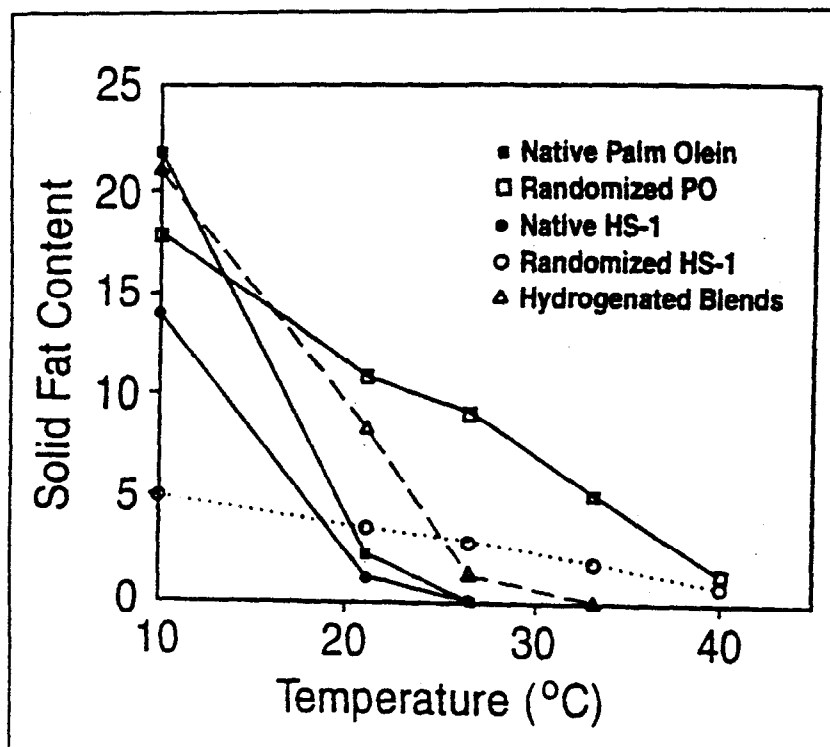
Figure 3. Graph plots for solid fat content with temperature for palm olein (PO) and high-stearate soybean oil (HS-1) before and after randomization.

either a SC-CO$_2$ or SC-C$_3$H$_8$ stream with a varying molar concentration of H$_2$ in order to conduct exhaustive hydrogenation of the FAME to fatty alcohols.

A response surface plot is shown in **Figure 4** for the hydrogenation of FAME as a function of reaction temperature and mole fraction of H$_2$ in the SC-CO$_2$ using a chromium-free catalyst. Under these conditions a maximum yield of fatty alcohols (C$_{16}$ + C$_{18}$ saturated alcohols from soybean oil) is attained at 250°C and 25 mole% H$_2$. Equivalent yields can be achieved using less H$_2$ in SC-C$_3$H$_8$ or by employing Cr-based catalysts and/or lower reaction temperatures. Gas chromatographic (GC) analysis of the hydrogenated products along with supercritical fluid chromatography (SFC) verified that the transesterification and hydrogenation processes were complete. As far as we know, this is the first case of a discrete two-step synthesis process conducted in critical fluid media that has the potential to be environmentally benign. Interesting recycle options also exist with respect to reusing the critical fluid media as well as the reaction by-product from the hydrogenation step, methanol.

velocities through catalytically-active, packed bed reactors.

We have taken their approach one step further by combining a transesterification reaction with a hydrogenolysis reaction using packed bed reactors coupled consecutively, and using either

SC-CO$_2$ or SC-C$_3$H$_8$ as a reaction and transport medium. Transesterification conditions similar to those quoted previously have been used to produce the FAME distribution that would be expected from soybean oil. These FAME are then introduced into

Recently we have also used binary critical fluid mixtures consisting of H$_2$ + CO$_2$ coupled with the traditional fat hardening technique. Elevated pressures of CO$_2$ and H$_2$ mixtures are used with stirred autoclaves to produce hardened fat compositions that have equivalent properties to margarine or shortening basestocks (**Table 2**). The mechanism for the hydrogenation of specific triacylglycerol moieties is a complex one, but suffice to say, addition of H$_2$ to CO$_2$ at 120°C results in selective hydrogenation in the range 50–100 psi (0.34–0.69 MPa), and the non-selective mode at H$_2$ pressures of 500 psi (3.45 MPa). As shown in Table 2, the
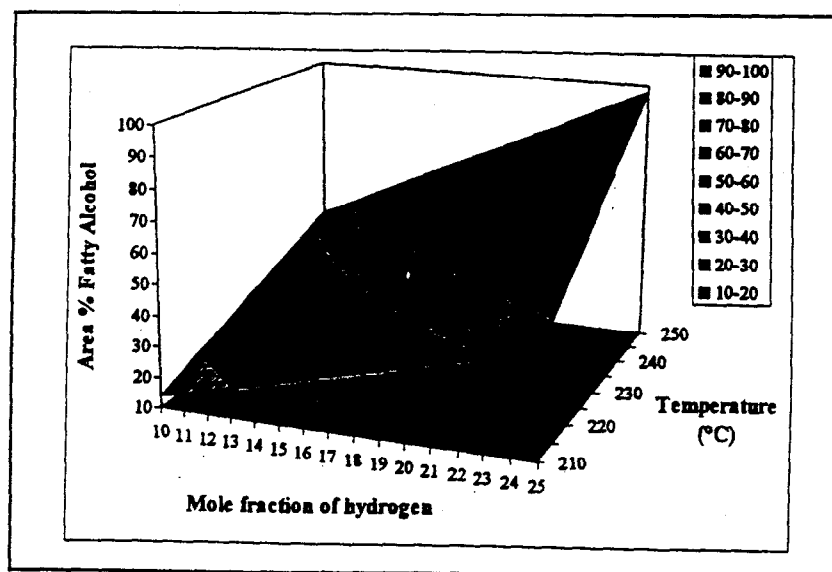

Figure 4. Response surface plot for the hydrogenation of fatty acid methyl esters to fatty alcohols in a system using hydrogen and supercritical-CO$_2$ with a chromium-free catalyst.

experimentally-produced margarine basestock substitute (the one hardened in the presence of H_2 + SC-CO_2) has a slightly higher iodine value (IV) and stearic acid content than its conventionally-produced counterpart; however the percentage content of *trans* fatty acids in the product hardened using H_2/SC-CO_2 is much lower than in the conventional product. Similar results were also found in comparing H_2/SC-CO_2 compositions with those used for shortening basestocks — the new method produced a healthier fat containing a lower percentage of *trans* fatty acids.

Subcritical water as an extraction and reaction medium for lipids?

Reference was made earlier to the use of subcritical water as a medium for processing lipid materials. Historically, subcritical water (not referred to by that name) has been used in the classical 'fat splitting' procedures, such as the Twitchell and Colgate-Emery processes. The temperatures employed for the splitting of fats must be high in order to ensure miscibility of the fat with the hot subcritical water. Under these conditions, a head of pressure is applied to the water to keep it in its liquid, or subcritical, state, between its normally atmospheric boiling point and its critical temperature (374°C).

As shown by others, varying the temperature on the pressurized water will change its dielectric constant and solubility parameters, making it more non-polar in nature and a better solvent for lipid moieties. We have exploited these properties recently for conducting the hydrolysis of model lipid compounds and vegetable oils in subcritical water at 250-340°C without the aid of a catalyst. Using a flow reactor composed of stainless steel (316 SS), we have achieved very rapid hydrolysis rates and high

Table 2. A comparison of the properties of a margarine basestock (dropping point 33–39°C) made using binary critical fluid mixtures consisting of hydrogen and carbon dioxide ('experimental') with one derived from the traditional fat hardening technique ('conventional').

Oil property	Margarine basestock	
	Conventional	Experimental
Stearic acid (%)	6–9	7–11
Trans fatty acids (%)	11–30	1–3
Iodine value (IV)	95–110	108–114

conversions of soybean oil to its fatty acid constituents. Yields exceeding 98% have been achieved in 10-15 minutes at 340°C and 13 MPa, using water-to-oil ratios from 5:1 to 2.5:1. Under these conditions, the water/lipid mixture is one phase as detected with the aid of a high pressure flow sight glass. There is some conversion of *cis* double bonds to *trans* but most products from this hydrolysis scheme are intended for industrial, not food, applications.

Conclusions

In summary, effective reaction chemistry and engineering of lipids can be accomplished in a variety of critical fluid media under environmentally-benign conditions. We hope that some of the described technology can be considered as a viable option that will assure compliance with environmental and worker safety legislation. The SFR option can also be coupled advantageously with extraction and fractionation processes conducted in critical fluid media to offer a completely 'green' approach to manufacturing, as well as being further economic rationale for the construction of these high-pressure processing facilities.

Bibliography

Andersson, M.B.O., *et al.* (2000) Synthesis of fatty alcohol mixtures from oleochemicals in supercritical fluids, *Green Chemistry*, 2, 230-234.

Jessop, P.G. and Leitner, W. (eds) (1999) Chemical Synthesis Using Supercritical Fluids, Wiley-VCH, Weinheim, Germany.

King, J.W. (1998) Sub- and supercritical fluid processing of agrimaterials: extraction, fractionation and reaction modes, in: *Supercritical Fluid — Fundamentals and Applications*, E. Kiran, *et al.* (eds), Kluwer Academic, Dordrecht, pp.451-488.

King, J.W. *et al.* (1999) Hydrolysis of soybean oil in a subcritical water flow reactor. *Green Chemistry*, 1, 261-264.

King, J.W. *et al.* (1998) Synthetic modification of seed-derived oils utilizing reaction chemistry in supercritical fluids, in *Advances in Oils and Fats, Antioxidants, and Oilseed By-Products*, S.S. Koseoglu *et al.* (eds), AOCS Press, Champaign, Vol.2, pp.314-320.

King, J.W (2000) Advances in critical fluid technology for food processing. *Food Science and Technology Today*, 14(4), 186-191.

Noyori, R. (ed.) (1999) Supercritical fluids, *Chem Revs.*, 99(2), 353-633.

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